ماجستير تناسليه (6)

Infertility

(Scheme for Evaluating)

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just print

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Scheme For evaluating sub fertile male

1. History
2. Clinical exam
3. Semen analysis
   - physical chics
   - mic exam
   - Biochemistry profile
   - sperm function tests
     - * sperm-cervical Mucus interact
     - * sperm Fertilizing Capacity

Lab

4. Endocrinal Evaluation
5. Genetic & chromosomal Evaluat°
6. Immunologic studies
7. Bact. exam → see Semen analysis
8. Radiological
   - ultra ls
   - Vasography
   - other
9. Histopath → Testicular Biopsy.

Clinical Exam.

(1). Gloved Fingers
(2). Complete undressing

1 General exam.

Look For

- Midline defects
- Breast
- Eunuchoidism Features

Eunuchoidism Features

① Skeletal
- Cranial to pubis / pubis to floor
- upper segment < lower
- Span > height

② Muscle mass ↓

③ Lack of virilize hair dist.
- Sparse axillary / pubic hair / facial / body
- Lack of Terminal Hair Recession

④ Infantile Genitalia:
- small: Penis, Testes, Prost.
- Undersdeveloped Scrotum.

⑤ High pitched Voice

- Muscle
- hair
- Voice
- Genitalia

Midline defects

Hare, lip or cleft palate
↓
Hypogonadism

Breast

Gynecomastia
① Testicular Tm
② Adrenal Tm
③ Liver dis etc...

Nipple disch. or Tenderness
↓
Prolactin secreting Pituitary Adenomas.

Examine ♂:

ⓐ * Heart & Lung
- Kartagner (situs inversus + immotile Cilia) → CF & Young

ⓑ * Abd
- Hepatomegaly
- Unreduced 2y Varicocele

ⓒ * Neurological exam
- Visual fields
- Reflexes.

2 Genital Examination

Penis

- Micropenis: if Hypogonadism before Puberty
- Hypospadias or epispadias
- Penile Curvature
- Nodules or masses (Psoriasis etc)
- Phimosis

Vas ∴ check for ← Whether nodules indurate

; CBAV 1-2% infert. & Ass e ← SV aplasia / Renal Anomalies (rare)

∴ thickened nodular vas → TB / previous Vasogram

Varicocele ① Large Varicocele → Can be seen thro' dilated scrotum

② Small Varicocele → distinct impulse & palpable dilated ↑ int. spermatic veins during Valsalva maneuver

③ If Varicocele detected → Supine ← Collapsed completely / No Collapse

Retro-peritoneal mass → Abd UIS

Cord lipoma.

Retroperitoneal vein thrombosis

NB! Varicocele in recent Abd. mass :

① Rt side
② not changed by Valsalva
③ not collapsed on Supine

Accessory → A. Prostate ①

(Circumferential movement ,)

② ← irregularities / Asymm. / Absent median Groove → S.P. in old age

↑ incid. of Cancer

Nodules

→ S.V. · NLy not palpated except if ← obst. / inflammed / Mid line cyst

(د. عبد الحكم) Endocrine Evaluation of Infertile Male

- **Incid** : ≃ 20% of infertility Cases ± oss e⁻ Endocrinopathy
- **Indications of Evaluation:**
  1. Azoo. or severe oligo (< 10 M/ml) (NB < OA: NL Hormones / NOA: Abn L ")
  2. Suspicision of Endocrinopathy e.g. ↓ libido & ED, Gynecomastia
- **Steps of Endocrine Evaluation:**

[A. History] [B. Exam.] [C. Invs] → Lab. Rad.

(A.) History: of.

- undescended testis.
- Delayed puberty
- ↓ libido, ED & Gynecomastia
- Diseases: Mumps, TB.
- Drugs: alcohols & Chemicals.

(B.) Examination:

[1.] General Exam:

- Eunchoid Features
- Cranial N. assesment in cases of pituit. Tm.
- Midline defect ??
- Gynecomastia
- Thyroid disorders

[2] local (Genital) Exam.

- see Kallman Syn.
- Testis size:
  - < 15cms → Hypog.
  - > 25 → Tm / Hydrocele

(NB) Hypogonadism:

- Cong. Small, Firm testis

Investigations

(1) Main Lab invs → Main Hormonal profile

- FSH →
- LH →
  - ↓ : 2ry Hypog.
  - ↑ : 1ry Hypog.
  - ↑ + ↑ T → Androgen insensitivity
- Testosterone
- prolactin

"A200" كتب كذا
- ↑ : 1ry Hypog...
- ↓ : 2ry ~
- NL ~

- Total. بتعلم منه
- Free T- & CFTR
- لما بتعلم كذا لا تروح

Estradiol: لمعرفة الحالة

(2) Other Hormonal invs: 4

(i) * Stimulat Tests:

to differentiate bet. Hypothalamic & pituitary Cause of 2ry Hypogonadism
- GnRH Stim. test
- Clomiphene Citrate or Tamoxifen test.

- GnRH Stim. test → لمعرفة مكان المشكلة
  - indicat: low NL level FSH & LH
  - Method:
    - (1st) 100 ug inj. of GnRH → 30-45 min
      - NLLY: ↑LH: 3 Fold the basal
      - ↑FSH: 1.5 ~ ~ -
    - So if:
      - No ↑↑ → Pituit. Cause
      - NL result → Hypothalamic cause.
    - to Confirm do:
    - (2nd) pump pulsatile GnRH test For 7 ds.

- Clomiphene Citrate test: use also to diff. bet Hypothalamic & Pituitary etiology.

- TRH Stim. test: to differentiate bet.
  - Hyperprolactinemia d.t
    - Tm Cause &
    - Non Tm " of pituitary

- IV TRH
  - ↑↑ PRL : Non Tm. Cause of Hyperprolactinemia
  - Sub NL ↑ PRL (< 30% of basal) ← Tm. cause.
- hCG stim. Test (؟؟ تزيد)

(ii) Inhibin B:

- secreted from sertoli cells in response to FSH.
- Regulate FSH sec. by -ve feed. back on pituitary
- shows: Circadian rythm (يعني في وقت بيقل ووقت بيزيد)
- ↓ level → sertoli cell dysf. → Impaired spermatogenesis.
- More sensitive than FSH in assessing spermatogenesis.

(iv) SHBG — اللي بيحمل التستوستيرون (TBG زي)

(v) Anti-Mullerian Hormone [AMH]:

- More sensitive & specific > hCG stim. test to differentiate bet. Cryptorchidism & Anorchia. [↓↓ level → Crypt.]

(vi) thyroid & Suprarenal Hormones.

(vii) DHT level, 5α-reductase & Androgen Receptor level (452 يسوى)

(i) Basics:
- FSH
- LH
- T (SHBG)
- Prolactin
- Inhibin B
- Estradiol

(ii) Stim. tests:
- GnRH ↑↑ Ant
- Clomiphene Cit. ↑↑ Ant
- TRH ↑↑ PRL
- HCG stim. " (& AMH)

(iii) Others:
- Thyroid
- Suprarenal
- DHT
  - 5α reductase
  - And. R level.

Genetic & chromosomal Evaluation of Infertile Male

① Incid:
② Indications
③ Types
④ Examination

Introduction

- Chromosomal failure or Abnormalities:
  - May be of: Sex chromosomes Abnormalities
    Autosomal " "
    - 2 (① Down ② Myotonic dystrophy synd) (other synds)

- Incid:
  - 6-13% of Infertiles
  - 10-15% of Azoo or Severe oligo pts.
  - 1% of Patients c (NL) Semen quality.

- Indications for chromosomal & genetic evaluation:
  - Azoo <
  - Severe oligo
  - +ve FH
  - DSD & PGD

① NOA [1ry (hypogonadism)
② OA c CBAVD → D of CFTR
③ severe oligo < 10 mill/ml (5-7% Azoospermia, 5-10% Cryptozoo.)
④ +ve FH of infertility (his brother)
⑤ DSD (Disorders of sexual Diff.)
⑥ PGD (preimplant. Genetic D)
⑦ Before ICSI or TESE / ICSI → (FISH) for Y-chromosome Microdeletion.

- Types of Genetic Testing

A Cytogenetic Investigations
1. Sex chromatin
2. Karyotyping
3. Fluorescence In situ Hybridization (FISH)
4. Comparative Genomic Hybridization [CGH]

B Molecular Genetic Investig.
1. Y-Chromosome Microdeletion
2. Sequencing of selected Genes.

[A] Cytogenic Evaluation 4/

① Sex chromatin.

[redacted] Barr Body)

[redacted] X-chromatin

Sample:

① [redacted]

② Vaginal cells

③ [redacted]

[redacted]

Plano-Convex, mass Continous ē the Nuclear memb.

[redacted] stained with [redacted] a stain"

- No of X Chromo-some = No of Barr Body + 1

Normal male (xy)	=	No Barr body
Normal female (xx)	=	1 Barr body
KF syndrome (xxy)	=	1 Barr body

Y chromatin Body (Y Body)

Buccal smear stained ē "quinacrin stain"

↓ exam. under Fluorscent MIC.

Potent Fluorscent spot (Y-body)

No of Y-body = No of Y-chromosome.

2. Karyotyping : (Chromosomal Analysis):

- done on peripheral Blood "Lymphocytes"
- Allow detection of "Numerical & structural" Chromosomal Aberrations

∴ WBCs : ++ → mitosis → arrest at metaphase → (Geimsa Staining) → detect Chromosomal abnormalities (2):

1. Numerical Abnormalities [aneuploidy]: extra or Missed Chromos.
2. Ultrastructural Abnormalities:
   - Translocat
   - Inversions
   - deletions

3. FISH.
4. CGH: For diagnosis of deletions or duplications of < 100 Kb.

B Molecular Genetic Investigations:

(1) Y-chromosome Microdeletion : by
- PCR
- Southern-Blot (SB)

(2) DNA Sequencing of specific Genes: done when specific genetic Mutation is suspected

e.g. - CFTR
- KAL1
- GNRHR
- GPR54

4. Examples of Genetic Infertility

Table 42.1: Genetic disorders causing male infertility.

Disorders of chromosomes

Numerical chromosomal anomalies

47,XXY (Klinefelter's syndrome and variants)

XX male · Down

XYY male

Structural chromosomal anomalies

Y chromosome microdeletions

Disorders of genes

46,XY disorders of sexual development (DSD)

- Disorders of androgen synthesis
  - Leydig cell hypoplasia, aplasia
  - 3β-hydroxylase / 17,20-lyase deficiency
  - 17β-hydroxy-steroid dehydrogenase deficiency [· 17β HSD
  - 3β-hydroxy-steroid dehydrogenase deficiency ·3β HSD]
  - 5-α reductase deficiency (5α red.)
- Disorders of androgen action
  - Androgen insensitivity syndrome (AR mutation)
- Other androgen disorders
  - Persistent Müllerian duct syndrome
  - Cryptorchidism
  - Isolated hypospadias

Disorders of extra-testicular ductal system

- Cystic fibrosis
  - CBAVD due to CFTR mutation
- Young syndrome

Disorders of the HPG axis

- Kallmann's syndrome and variants
- Isolated FSH deficiency
- Isolated LH deficiency

Structural sperm defects

- Globozoospermia
- Primary ciliary dyskinesia (Immotile cilia)

Inheritance نوع كل (AR, AD, XL)

Disorders of

- Chromosomes
  - Numerical
    - KS
    - XX male
    - XYY
    - Down
    - Norman
    - Mixed gonadal
  - Structural
    - AZF
    - MiCnd.
- Genes
  - (i) AKS
    - Kall. Synd (AR, AD, XL)
    - AR or AD ← Prader Willi
    - Isolated → LH / FH
  - (ii) duct system
    - CF (CFTR) [AR]
    - Young Synd
  - (iii) Sperm defect
    - (AR) · Immotile cilia
    - · Globozoosp.
  - (iv) DSD: (Ambig.)
    - AIS
    - Persistent MDS
    - Crypto
    - Isolated Hypospp.
    - 5 α reductase def.

AR (PIK1) gene.

Radiological Evaluation of Infertile Male

(A) Ultrasonography

- Scrotal (US / Doppler)
- TVUS / TRUS

① Testicular findings:
- Testic. Size (↓ atrophy)
- Cryptorchidism (↓ atrophy)
- Varicocele (↑ atrophy)
- Hydrocele
- Orchitis] ↑ echogenicity
- Torsion]
- TMS → (US Findings ±)

TRUS Value
- SV disorders: aplasia / hypoplasia & dysf.
- Prostatic disorders:
  - Prostatitis
  - BPH
  - Calculi / Cysts
  - Follow up during T.
  - Cancer.

② Epididymal & Vasal Finding
- Size: Caput: 10 mm; Tail: 5 mm.
- (i). Epid.:
  - Cyst (NL in 70%)
  - Spermatocele
  - Inflammat°: Acute: ↑ Echogenicity; Chr.: ↓ Echogenicity
  - Obst.
- (ii). CBAVD

(B) other Imaging: Vasography; Abd. & Thyroid US; CT & MRI ??

(C) ~~PPDU~~ (penile Duplex).

NB

Colour Doppler ultrasound of the scrotum can detect a varicocele in around 20% to 30% of infertile males. This part of the investigation should also be performed in a standing position. Accepted ultrasound criteria for the diagnosis of a varicocele is a venous diameter >3 mm with or without Valsalva maneuver, an increase of venous diameter during Valsalva maneuver, and venous blood flow reversal (reflux) for >2 seconds.

- On the basis of the amount of reflux present, varicoceles can be graded as follows:

- Grade I, slight reflux (<2 seconds) during Valsalva
- Grade II, reflux (>2 seconds) during Valsalva, but no continuous reflux during the maneuver
- Grade III, reflux at rest during normal respiration or continuously during the entire Valsalva maneuver

Testicular Biopsy

Indications : [1] in Cases of Azoospermia

NOA
(Normo- or Hypergonadotropic Hypogenadism)
↓
as a therapeutic (for TESE) & Diagnostic (SCOS)

OA
(NL FSH < 7 IU/L, NL Test. Vol, ↓ Markers)
↓
to confirm NL spermatogenesis before surgical Correct of obst. (this doesn't apply for pt ē CBAVD or vasectomy; unless ↓ Test. Vol or ↑ FSH) [EAU 2016]

[2] **Diagnosis of CIS : in high risk Conditions :**

- Infertility
- Cryptorchidism
- TGCT (test. Germ cell Tm)
- Idiopathic Testicular atrophy
- Some CIS Findings (risk of CIS) :
  - Microlithiasis (if > 20)
  - "not Homogeneous" → inhomogeneous test. parynchyma
  - Sfid testicular lesions.

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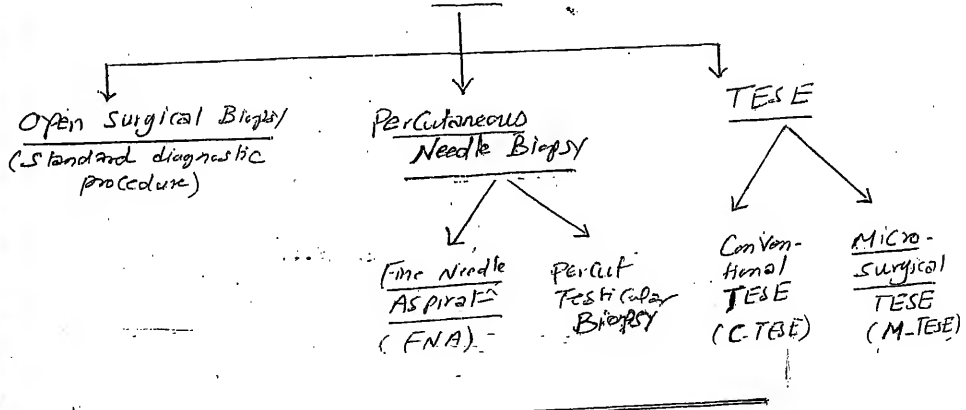
so; Testicular Biopsy has 3 Main indications :

[1] **Diagnostic** : for Diagnosis of **CIS** & NL Spermatogenesis before Correct of OA.

[2] **therapeutic** : TESE in Cases of NOA

Types of Testicular Biopsy



1) Open Surgical Technique:

1. Anaesthesia { General / spinal or / Local.
2. غسل الخصية بالبيتادين ثم شد الخصية لأعلى وتحلل الـ epididymis بعيد عن الـ post. "Incision line"
3. 1-2cm Incision ب { Skin / T. Vaginalis } → Exposure ل T. Albuginea.
4. 0.5 cm Incision ب T. Albugina: light Testicular pressure → Testicular Tissue Extrusion يتم Excised ب sharp scissor (Biopsy should be 3x3x3 mm; containing at least 1:0 SNT).
5. Tunica Albuginea is closed ب 3/0 chromic Cat. gut Then Scrotal skin Suturing.
6. Proper Hemostasis ب cold Compresses.

Handling of the Specimen.

↓

Microscopic exam.

1st part: Cytological Examination using Wet prep. or Squash prep technique (Fresh Mic. exam).

Specimen + drop of saline or Ringer → Squash (not applied)

Specimen under Cover Slip & Examined by Phase Contrast MiC. → if there is Sperm Specially if Motile → look. obst. (2) ICSI or Cryopreservation

2nd part: Histological Examination after Fixation by either

Fixation staining

↓

- Bouins sol or
- Steve's sol (formaline is contraindicated)

↓ then:

Staining By: either:

- H & E
- Pap
- DiffQuick

↓

- 4 Main Findings (1-4)
- 2 other (5, 6)
- Johnson-Stirling.
- CIS (99)

evaluate ← spermatogenesis, CIS.

Interp- relat^n & Biopsy

① WL Spermatogenesis:

- S.F.T diameter → 150 - 300 μm & Contain all stages & Germ cells.
- Spermatogonia & Sertoli PAS (Rest on BM

② Hypospermatogenesis:

- ↓ Germ Cell No
- Some mature Sperms & Spermatids present (NL Sepermatogenesis)

③ Spermatogenic arrest:

- arrest of spermatogenesis may occur at Stage of < 1ry spermatocyte or Spermatid,

④ Sertoli Cell only Synd. (SCOS).

- only Sertoli cells present ; complete absence of Germ cells.

⑤ Premature Separation or Sloughing:

- Premature Separation & sloughing of Spermatocytes into the central lumen of Tubules. e.g Varicocele. ??

⑥ Peritubular Fibrosis & Tubular Hyalinizat^n.

- Thickening & Seminiferous Tubules walls & Hyalinizat^n → Germ Cell loss.

Seen in < Irradiat^n. / Klinefelter / Infect^n.

Scoring Method (Johnson's Method)

Transverse Sections of Tubules are Selected in each Biopsy & Cells within them are Counted:

↓

Johnson Scoring.

10 → NL Spermatogenesis:
- Complete "
- many Sperms
- Germinal epithelium organized into regular Thickening → Open lumen.

9 — Germinal epith. disorganized ē < Sloughing or obliterated lumen
Many Sperms. ↓

8 Few Sperms (5-10)

7, 6, 5 — No Sperms but < many Spermatids [7] / few " (5-10) [6] / No Spermatids but [5] Spermatocytes.

4 Few Spermatocytes only (<5)

3 only Spermatogonia

2 SCO (No Germ Cells)

1 No Cells in Tubules (Complete hyalinization)

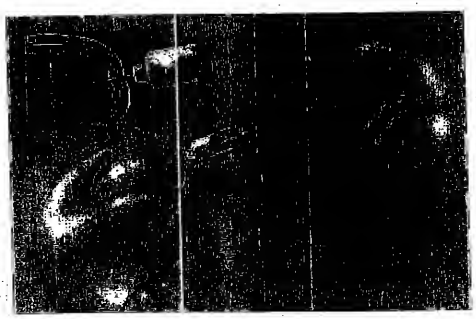
Total Score = Individual Score / No of Examined Tubules.

NB: the open diagnostic biopsy has several **limitations.** For one, it is invasive. Second, it only provides information on the area that is biopsied and tells us nothing about sperm production in the rest of the testis. Third, how clinicians read the biopsies varies widely, making the interpretation unclear, a fact that does not help the patient

2- Percutaneous needle biopsy:

A- *Testicular Fine Needle Aspiration (TFNA)*

- ***Technique:*** using a small butterfly needle attached to a syringe, may also be used to harvest spermatozoa for ICSI, especially in men with OA.



- ***Advantage:***

1- Does not require surgical equipment and experience.

2- Less risky and painful than open biopsy

3- Can be performed in an outpatient setting under local anaesthesia.

4- A correlation of 88.5% between fine needle biopsies and normal histology in different patient groups.

5- FNA may also be helpful in the diagnosis of small testicular lesions. It is, however, unclear if FNA can also accurately detect CIS of the testis.

6- Very successful in severe hypospermatogenesis

- ***Disadvantage:***

1- Associated with a lower sperm retrieval rate in men with NOA compared to open biopsy techniques.

2- Cryopreservation is not appropriate for sperms obtained by it due to very low number and grater blood contamination..

3- Doesn't allow histologic examination (so of limited value in diagnosis of CIS).

- ***Complications (rare)***: testicular hematocele, hematoma, epididymal trauma (as it is a blind technique).

Until standards for the evaluation of aspirated material are well established, open testis biopsy is the diagnostic procedure of choice. Fresh unfixed testis biopsy materials should be examined in the operating room to determine sperm are presented and whether they are motile.

B- Percutaneous testicular biopsy :

Percutaneous testis biopsy using a Tru-Cut type of devise has been performed as an office procedure under local anesthesia. It has been used fo evaluation of both histology and cytology. This blind biopsy procedure could result in unintentional injury to either the epididymis or testicular artery coursing under the surface of the tunica albuginia. In additiona, we have often found that sepcimens obtained in this way often contain only three to six tubules with poorly preserved architecture. Specimens obtained in this way can be used to extract sperm for ICSI in case of obsteruction

Sperm Retrieval in Azoospermia

Azoospermia

- **Obstructive (OA)**
  - Functional OA → Sympathetic injury
    - Mild → RGE: See semen Retrieval & Surgical
    - Severe → Failed Emission:
      - Electrical therapy
      - Prostatic Massage
      - Surgical Retrieval
  - Organic OA
- **Non-obstructive (NOA)** → TESE أو أخذ
  - or
  - TFNA
  - open surgical Biopsy

Surgical Sperm Retrieval in Cases of OA

- TESE
- MESA
- PESA
- MVSA

- **Testicular Sperm Retrieval**
  - FNA
  - TESE — C-TESE / M-TESE
- **Epididymal Sperm Retrieval**
  - ESA (Epid sperm aspiration)
    - Microsurgical (MESA)
    - percut. (PESA)
- **Vasal Sperm Retrieval**
  - Seminal Tract Washout (STW)
  - Micro-surgical V def. Sperm aspirat (MVSA).

Testicular Sperm Retrieval

(1) TeFNA see before.

(2) TESE.

- Indications: NOA (..Cryptozoospermia) / OA (if MESA Failed)
- Types: A. Conventional TESE (C.TESE) — B. Microsurgical or Microdissect TESE (M-TESE)

- Considerations before TESE:

1. doing it at same day of oocyte retrieval (to maximize the potential to retrieve viable spermatozoa for use in ICSI)
2. should be delayed 6ms after any Inguinoscrotal Surgery or Testicular Biopsy
3. Multiple Biopsies Should be avoided To ↓ risk of Testicular devascularization
4. Use of optical magnification (↓ Testicular injury).

- Conventional TESE Technique:

Technique

TESE-either as a single extraction *(single TESE)* or as multiple extractions from different areas of the testis surface *(multiple TESE)*-may be performed under a local anesthetic using one of the following techniques:

1. Complete test. exposure & scrotal exploration → Dx of OA reanalization
2. Window Technique.

Exposing the testicle completely, along with scrotal exploration in case of suspected OA, to evaluate the presence of dilated epididymal tubules and the possibility of surgical re-canalization (tubulovasostomy) to be performed at the same time;

Using the "window" technique, i.e., performing a very-small longitudinal or transversal incision of the scrotum.

In both cases the surgical steps are: opening the tunica vaginalis, performing a transverse albuginectomy of about 5 lo 10 mm, forcing out and excising a small quantity of testicular tissue, controlling hemostasis (bleeding mainly comes from the sub-albugineal tiny vessels), closing the albuginectomy, the tunica vaginalis, the dartos, and the skin.

Avoiding touching the testis surface with gauze and infusing 1.5 mg of betamethasone solution inside the vaginal cavity, while ending its reconstruction, prevents adhesions with the albuginea from forming, making repetition of the procedure or subsequent surgical re-canalization easier.

Biological preparation of the removed tissue is the same as for Micro TESE.

• If by Examnat :

↓

No Spermatozoa : then

(1) Additional Biopsies are done From the same Tunical Incision

(2) Biopsies From additional Incisions

(3) Central Biopsies are obtained.

↓ then

Testicular tissue processing : (Microdissect)

(1) Cryopreserve it

(2) Histopathological Exam. ?? because

. Carcinoma in situ affect upto 1.1 % of infertile men

. Seminoma affect 2% of Cryptorchidism

Results

The retrieval of testicular spermatozoa in cases of NOA is significantly better-quantitatively and qualitatively-with TESE than with TeFNA. ***TESE is the recommended procedure to retrieve spermatozoa in NOA patients***, yielding sperm for ICSI in 52.2%

compared to 23.0% by TeFNA. In these patients a high sperm recovery rate is achieved even when repeating TESE. ***Multiple TESE would appear to improve the success rate compared to single TESE*** (52.5%).

Complications

The very rare complications of TESE are those common to any small surgical procedure: infection and bleeding with scrotal hematomas that rarely require surgical drainage. In cases of NOA patients with very small testes, testosterone deficiency following surgery must be considered.

Micro-dissection of testicular tissue (Micro TESE)

Introduction and indications

In an effort to increase the chances of finding islands of spermatogenesis in sampled tissue, micro-dissection of testicular tissue (MicroTESE) was devised. This techniques, involving book like opening of the testis followed by a careful search fur suitable tubules using an operating microscope, allows the surgeon to recover sperms in some "difficult" cases of NOA.

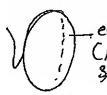
Technique

MicroTESE involves "bivalve" opening of the testicle by means of an equatorial or longitudinal incision under general or spinal anesthesia and removal of single tubules observed to have the largest diameter under an operating microscope or, in, the absence of larger tubules, of those closest to vessels and at different depths in the pulp ***(testicular mapping)***.

The surgical steps are as follows:

1. An equatorial incision is performed under general anesthesia along three-fourths of the circumference. A relatively avascular albugineal line is selected for this purpose. Micro-coagulation of the few bleeding sub-albugineal vessels is performed by a bipolar thermal device.

2. Testicular lobules are carefully separated. ***Individual seminiferous tubules are then extracted*** from either side. ***About 30 testicular draws are usually obtained from each testis.*** Micro-dissection is performed with 18 x to 24x optical magnification.



3. At the end, testicular pulp is gently compressed by gauze for 2' to ensure hemostasis. The tunica albuginea is then closed with a Vicryl 5-0 continuous suture, followed by closure of the tunica vaginalis and infusion of a corticosteroid solution inside its cavity, and by dartos skin closing.

The fragments of testicular tissue (TESE) or extracted tubules (MicroTESE) are put into a Petri dish, in 2 mL HTF medium. Careful fragmentation of the tubules by tiny scissors is performed, and the fluid is passed through a 24-G angiocatheter several times, until a cloudy suspension is obtained. At the end, the fluid is microscopically examined to detect spermatozoa and other germ cells.

Results — TESE : 37 %
M. TESE 54 %

MicroTESE may increase positive retrievals in NOA subjects (54-63.4%), and a revious failure with TESE does not exclude a successful MicroTESE. In fact, successful MicroTESE retrievals were reported even in the worst histological conditions, such as Sertoli cell-only syndrome (SCOS). Compared with TESE, MicroTESE was reported to achieve higher success rates (54.6% versus 35.7% in a meta-analysis) and had significantly more effective results in patients with high follicle stimulating hormone (FSH) levels; therefore, at least in these patients MicroTESE should be the preferred choice.

Complications

With MicroTESE, less testicular tissue is removed, thus greatly reducing the risk of endocrine deprivation. Moreover, there appear to be significantly fewer vascular complications than with TESE; at six-month ultrasound follow-up no parenchymal or vascularization abnormalities were reported.

Predictive factors of sperm retrieval in nonobstructive azoospermia

The only good predictor of successful retrieval is testicular histology, which is unfortunately the least useful predictor for clinicians, since the histological sample is usually obtained at the same time as TESE.

No clear relation was found between successful sperm retrieval and serum FSH levels or serum inhibin-B levels or testicular volume; seminal plasma inhibin-B was reported as an independent predictor of a

1. Test. Histology, PX
2. ALP a or b
3. No relat- e T. Vol FSH Inhibin- B.

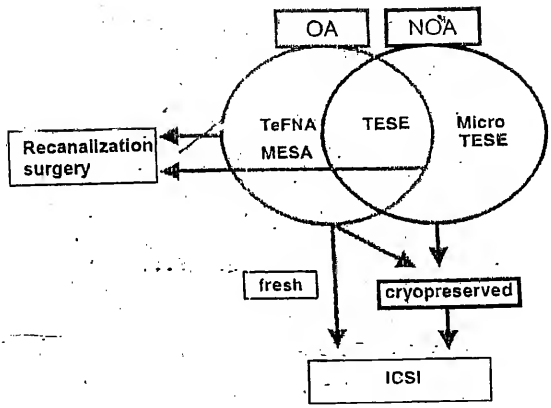


Fig. 62.1: A flowchart for treatment of azoospermia.

Figure 62.1 summarizes a flowchart for treating azoospermia.

Recommendations

A testis biopsy aimed to differentiate OA from NOA is indicated only in azoospermic patients with normal orchidometry and normal FSH.

In OA due to epididymal obstruction (CBAVD excluded), MESA and/or TESE and sperm cryopreservation should be carried out together with a microsurgical seminal tract re-canalization.

In NOA, TESE (either single, multiple, or microsurgical) should be used rather than TeFNA due to their quite different chances of successful sperm retrieval.

In NOA with very high FSH, microsurgical TESE should be preferred.

In NOA sperm cryopreservation should follow any successful TESE procedure.

4. Chromatin Studies

(NL ؟) (Sperm DNA Damage)

NL DNA of Sperm Means:

- No DNA fragmentation, oxidation (Nor) denaturation.
- Chromatin is: Stable or Condensed (NL Packaging)
- Histone replaced by Protamine (NL Cells: Histones / Sperm: Protamine)

So Sperm DNA damage Means: DNA Fragmentation / Abnl Chromatin Packaging &/or Protamine deficiency.

→ Failed union bet ♂ & ♀ Gametes → No fertilization

[Sperms of infertile men have DNA damage > Fertile]

Causes of DNA damage:

Intra testicular (Iry testicular) causes.

- Genadotoxins
- Aging
- ↑ ROS e.g. infection
- defective spermiogenesis (Protamine deposition occurs during this stage).

Extra testicular (External) Causes

- Chemotherapy
- Radiotherapy
- Smoking
- Varicocele
- Genital tract inflamm.
- Hyperthermia.

Effect of DNA damage on reproductive outcome:

① on Vivo fertilization :→ ↓ pregnancy rates via intercourse or IUI or Recurrent loss.

② on Vitro Fertilization: no effect neither on Fertilization rate of IVF/ICSI nor embryo development

2. Culture studies

may be needed for:

Semen

- If there is evidence of infection or inflamm.
- e.g. Round cells > 1 million/ml (WBCs) or ≥ 10 / HPF

Urine

- If there is evidence of urethritis or cystitis, prostatitis.

3. Chemical studies

(optimal tests to study)

Chemical markers:

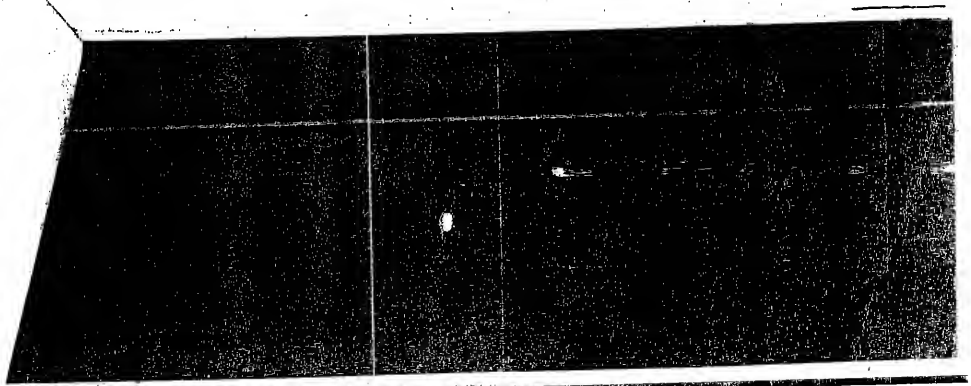
- Epididymal markers ..
- SV markers:
- prostate Markers:

(3) ?? epi → α glucosidase
(3) SV → Fructose
(4) prost → Citric acid.

Spermfunctions

By estimation of "ROS" level

- origin:
- Level:
- Examples
  - NO
  - H_2O_2
  - Hydroxyl Radical
  - Hydro/peroxyl



ROS (reactive oxygen species) & Infertility

- **Def. of ROS**: highly reactive oxidizing agents belonging to class of free radicals. a free radical is "any atom or molecule that possis one or more up-paired electron"
- **Types of Free radicals**: H_2O_2 : Hydrogen peroxide
  - .OH : Hydroxyl Radical
  - $.O_2^-$: Super-oxide anion
  - ROS ← Reactive Nitrogen Species (RNS)
- **Sources of ROS**
  - WBCs (main source)
  - Sperm (NL & abNL)

Effects (Functions) of ROS

Beneficial Effects:

Small amount of ROS can regulate sperm capicitation, ARV membrane Fusion.

Harmful Effects

High levels of ROS → Oxidative Stress: ⇒ may → sperm dysfunction & death. 2 Terato Zoo. by ① ②

Oxidative Stress Means:

1. **Lipid Peroxidation**: oxidation of unsaturated free fatty acids → Cellular dysfunction
2. **DNA damage**

Natural Defence Mechanism against ROS
(ROS scavengers or antioxidants)

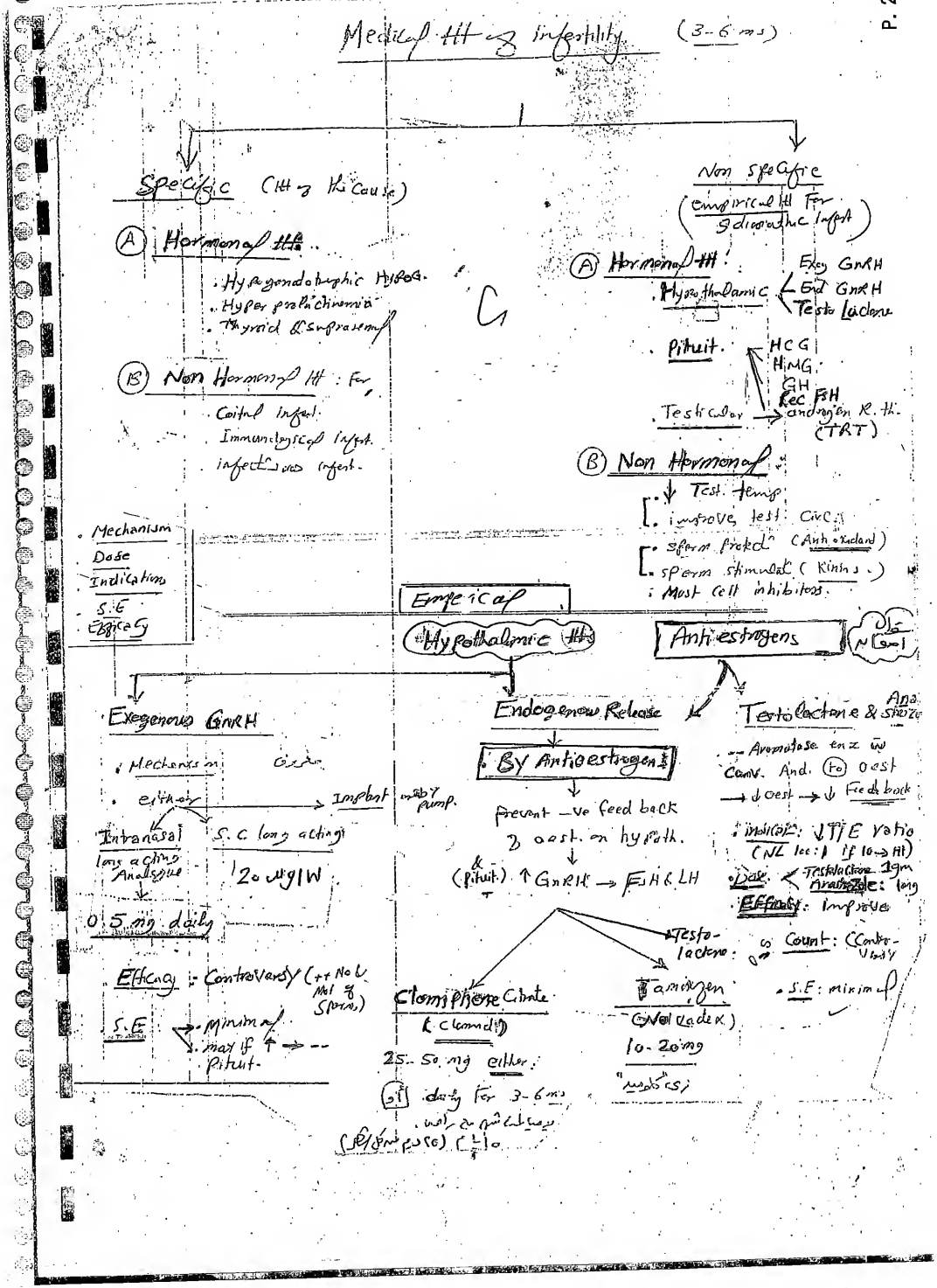
- **Enzymatic antioxidants:**
  - Catalase
  - Superoxide dismutase (SOD)
  - Glutathion peroxidase (GP)
- **Non Enzymatic anti-oxidants**
  - Vit E
  - Vit C
  - Uric acid.

the scavengers are present in semen in specific level. if the balance bet oxidant (ROS) & anti oxidant (scavengers) disturbed ⟶ oxidative stress.

- **ROS & infertility:**
  - in fertile men ⟶ ver low amount of ROS
  - in infertile men (25%): ⟶ High level of ROS ⟶ usually caused by leukocytospermia.

- **Lab assessment of ROS:**
  - ① Chemiluminscences.
  - ② Cellular probes coupled ē flow cytometry.

NB) ROS ⟶ Teratozoospermia [Excess Residual Cytoplasm] (ERC)


Medical Hlg infertility (3-6 ms)
Specific (Hlg the Cause)
Non specific
(empirical Hlg For Idiopathic Infert)
A Hormonal Hlg
. Hypogonadotrophic Hypog.
. Hyperprolactinaemia
. Thyroid & Suprarenal
B Non Hormonal Hlg: For
Coital Infert.
Immunological Infert.
Infect's orig Infert.
A Hormonal Hlg
Hypothalamic
Exog GnRH
End GnRH
Testo Lactone
Pituit.
HCG
HMG
GH
Rec FSH
Testicular
androgen R. Hlg (TRT)
B Non Hormonal
↓ Test. temp
improve test. circ.
sperm protect (Anti-oxidant)
sperm stimulat (Kinins)
Most cell inhibitors
Mechanism
Dose
Indication
S.E
Efficacy
Empirical
Hypothalamic Hlg
Antiestrogens
Exogenous GnRH
Endogenous Release
By Antioestrogens
Testolactone & Anastrozole
Aromatase enz in Conv. And. to oest
↓ oest → ↓ Feedback
Indicat: ↓T/E ratio (NL 1cc:1 If 10→ Hl)
Dose: Testolactone 1gm Anastrozole: 1mg
Efficacy: Improve
Testo-lactone
Count: Control-Unsatisfactory
S.E: minimal
Clomiphene Citrate
(Clomid)
25-50 mg either
or daily for 3-6 ms
Tamoxifen
(Nolvadex)
10-20 mg
Mechanism
Dose
Indication
S.E
Efficacy
Mechanism
either
Intranasal long acting Analogue
0.5 mg daily
S.C long acting
20 μg/IW
Implant by pump
Prevent -ve feed back
↓ oest. on hypoth. & (Pituit.) ↑ GnRH → FSH & LH
Efficacy: Controversy (++ No L Mot of Sperm)
S.E: Minimal
may ↑ → -- Pituit.

(NB) Clomiphen & Tamoxifen → ↑ FSH (↑ spermatog.) & ↑ LH (↑ libido)
↑ testost: ± ← Axis
↑ peripheral conv. & in liver to Oestrogen
Monitor Test. & Estradiol level.

Efficacy of Clomiphen & Tamoxifen
① Improve Count → Improve Count Rather than Motility
② # of Hypogonad. Hypog
③ # of Partial Androgen Resistance (PAIS)
④ NoA before TRE → TW a Course.

• Clomiphene Citrate S.E: (5%)
• Nusea, Vertigo, hair loss, Visual disturbances } → Stop
Following: T, E2, BP
• Suppression of Spermatogenesis (d.t weak Estrogenic Activity especially in large doses (200–400 mg/d))
• Wt. gain or loss.
• HTN
Paradoxical Azo

• Tamoxifen S.E: as Clomiphene but:
① less common S.E
② weak intrinsic estrogenic activity → less incid. of Paradoxical Azo.

Pituitary Hs (3)

HCG
Mech. → has LH activity & (weak FSH activity)
Dose → see Hypogonadotrophic Hypog.
Indications:
• Hypogonadotropic Hypogonadism
• Normo N°
• Post Varicocelectomy if Count < 10 million/mp.

S.E: (↑ androgen): ① Acne (precocious Pub.) ② behaviour & libido change ③ Gynecomastia

• Regimen: start 1500 IU Twice IW for 6 ws & if No ↑ T. after that → 3000 IU Twice IW.
(resp.) (1500–3000 IU Twice IW).

Menotropins HMG
Mech. → has FSH activity (&
dose → see 75–150 IU 3 times IW
Indications: as HCG
Effect in pt. NL Gns. level → Controversy

• Purified FSH (Genuf F) 150 IU/d for 3 ms.
→ NO significant improvement in Semen Parameters or Pregnancy Rate
→ IVF: Improved outcome in those NL conventional Parameters.

Growth Hormone HH

Mech. ① Direct stim of Leydig cells.
② Release of Insuline like growth factor 1 (IGF-1)
++ on Leydig; on Sertoli (IGF-1) From Sertoli Cells → effect spermatogenesis.

dose: Nordittropin 2-6 IU S.C.

Efficacy: improvement in Semen parameters & pregnancy rate (Controversy)

S.E: ① Paraesthesia of fingers.
② Joint swellings.
③ ↑ Liver enzs.
Reversible

Non Hormonal HH

↓Scrotal Temp (Scrotal Hypothermic device)

- Effect of High temp on testicular growth → (see varicocele)
- it was found that (85%) of pts with Idiopathic infertility having ↑ intrascrotal Temp
- the patient wear a device around the scrotum → ↓ scrotal temp by water evaporat → improvem. in Semen Parametes & Preg. rate.

Improving Testicular Circulat

↓

2 drugs

① Pentoxyphylline (Trental) / (Pentoxifylline)
② Trazocin HCl (Hytrin)

Pentoxyphylline (no benefit in ...)

Methyl Xanthine derivatives ≤ Caffein / Theophyllin → PDE → ↑cAMP

used in Hig Idiopathic infertility ← { ① Improve testicular & Epid. Microcirculat° (by ↑ RBCs Flexibility) ; ② ↑ Sperm Hyperactivat° & motility (↓OAT)

Cevidox 400 (Trental 400) → dose 1200 mg/d.

Efficacy → many studies showed improvem. in count, Motility & Morphology of it (Improve OAT)

SE → mild (nausea & drizz.)

Trazosin Hcl (Hytrin)

(i). α-Blocker → Arterial wall Relaxat° → improving testic. Circulat° & Function.

(ii). 1-2 mg/d (SE < Hypotn dizziness)

Sperm stimulation

(1) Kallikreins: ↓ Convert Kininogen to Kinins

(2) ACE I ↓ Kininase enz ↓ ↑ Kinins

(3) Indomet. Cin & Ketoprofe, 5-4 ...

. Kinins play a role in Sperm Motility & Migrat° through Cervical Mucous.

SE ↓ Exacerbat° of Genital Inf..

Dose: Kallikrein enz. (600KU/d) (Padulin) ®

• Sperm protect ⊃ (Antioxidants)
- Antioxidants
- Zinc
- L-carnitine.

① Vitamins ⊂ A, E, C ⊃ Pentoxifylline, Allopurinol, Glutathion & Selenium
all ↓ ROS ↑ is usually higher in 40% of infertile

NB: Vit C level in smokers semen is low
- No Controlled studies Confirmed the Effecacy.
- 100-mod doses → are Needed while high doses may have adverse effects as ROS may be Needed for AR.
- Glutathion: 600 mg EOD (IM) for 4 mo → improve semen parameters but not pregnancy rate

② Zinc:
- Zinc NLLy present in semen, secreted from the prostate.
- Exogenous Zinc ± < ↑ T. level, ↑ sperm product.
- Zinc Supplemental in: < NL level pt → little Benefit; Malnourished pt → ↑ Benefit.
- Dose: 25 mg/d (Higher doses are harmful).

③ L.Carnitine:
- Infertile Men I have lower levels.
- May improve the Motility [Controversial Results]. ✓

Dose: 2-3 gm/d.

Mast Cell Inhibitors

- Mast cells present in human testis & peripheral tissues & play important role in → inflamm. / Hypersensitivity / Fibrotic conditions.
  - By Histamine & seretonin → Fibrosis → altered spermatogenesis

- Mast cells & ♂ Infertility:

① Mast Cells → Histamine & Serotonin → role in Steroidogenesis.

② Mast Cells → ++ Fibrosis → disturbed spermatogenesis (↑ testicular mast cells ass. ē ↑ Fibrosis ω is common in pt. ē Infertility).

- Ebastine & Tranilast

↳ Mast cell blockers ± play role in ♂ Male infertility

in 2 studies

- Tranilast (Anti-allergic)
  - ↓
  - in 50 pts ē oligozoo. (Controlled Randomized study)
  - ↓
  - Significant higher level ♂ semen parameters &
  - 28.6 Pregnancy Rate

- (ايبستين) Ebastine
  - ↓
  - 15 Idiopathic oligozoo. patients:
  - ↓
  - * 66.7% definite improvement in Semen quality
  - * 20% Pregnancy Rate

1. Testosterone Replacement Therapy (TRT)

- Indications
- Formulations
- SE
- CI
- Monitoring
- Efficacy
- others

a. **Clinical applications:**
   - Male hypogonadism (main use).
   - Delayed puberty.
   - Micropenis.
   - Female-to-male trans-sexuals.
   - Aplastic and renal anemia.

b. **Controversial application:**
   Senescence. (الشيخوخة)

c. **Experimental use:**
   - Excessive growth.
   - Male contraception.

Ob. d. **Obsolete application:**
   Idoiopathic infertility (as an empiric therapy).

Ab e. **Testosterone abuse:** (إساءة استعمال).
   High-performance athletics and bodybuilding.

(B) Testosterone Replacement Products

Formulation	Dosing Ranges	Advantages	Disadvantages
Oral Formulations → See below			
Injectable Testosterone enanthate or cypionate	100 mg/wk IM or 200 mg every 2 wk IM	Improves symptoms, inexpensive, longer intervals between dosing	Requires injection; fluctuations in serum testosterone levels
Topical gels (Testogel) ® (Abd. & Upper arm)	Gel sachet (50–100 mg testosterone) applied daily	Corrects symptoms, flexible dosing, ease of application, good tolerability	Potential for secondary exposure
Transdermal patches[a] (Androderm) ®	1–2 patches (5–10 mg) every 24 h	Ease of application, corrects symptoms, mimics diurnal rhythm, less erythrocytosis	Lower serum testosterone levels achieved, skin irritation likely
Buccal tablets (buccal mucoadhesive system)	30-mg controlled-release tabs applied twice daily	Corrects symptoms	Gum and mouth irritation
Implantable pellets (at abdomen)	4–5 pellets (each contain 200 mg) implanted every 3–6 mo	Corrects symptoms, long duration of activity	Requires surgical implantation; pellet extrusions, infection

- Under trials: sublingual T.

NB: patches, gels

- oral Formulations: enanthate, cypionate
  - old Alkylated Formulations
    - 17α-methyl-Testosterone
      - S.E: Hepatotoxicity, Hepatocellular Carcinoma.
  - New Formulations
    - Testosterone undecanoate
      - Safe (No hepatic S.E).
      - Dose:
      - disadv: Poor or variable absorption, Substantial Hepatotoxicity.
  - DHT derived oral Preparat
    - Mesterolon
    - 17 MENT

(C). Side Effects of TRT:

- Gynecomastia
- Polycythemia
- ↑ wt gain
- Axis suppression.
- Flaring up of Cancer prostate
- Worsening of BPH
- Acne
- Aggressiveness
- Premature Epiphyseal Closure
- Liver Toxicity.

(D.) Contraindications and precautions for testosterone replacement therapy[2]

Contraindications (Absolute)	
• Male breast cancer	
• Prostate cancer (known or suspected)	• Sleep apnea.
• Hypersensitivity.	
Precautions (Relative CI)	

- Gynecomastia
- Hyperlipidemia
- Erythrocytosis,
- Azoospermia or Test. Atrophy
- BPH
- Hepatic dysfunct
- CVS dis. (CHF, severe HTN, peripheral Edema)
- Polyglobulism.

(E) Monitoring of TRT.

1. prostate — PIR, PSA, TRUS → in any pt >50 y.
2. Bone: Bone Mineral density (BMD) of Lumbar spine & Femoral Neck & epiphyseal Closure.
3. Haematocrit value
4. Testosterone level
5. lipid profile
6. 3 parameters (BY History)

- Somatic
  - Body Proportion & wt
  - Ms: mass & strength
  - Hair, voice & sebum
- Sexual
  - Libido
  - Erect
- Psychic
  - Mode
  - Well being
  - Intellectual Activity

(HL) NB **Endocrine Society Guidelines for the monitoring of testosterone therapy**

	Start of treatment (baseline)	Each visit	3 months	Annually	1–2 years
Symptom response		√	√	√	
Adverse events		√	√	√	
Formulation-specific AEs		√			
Testosterone levels	√		√		
Haematocrit*	√		√	√	
BMD of lumbar spine/femoral neck†					√
DRE‡	√		√		
PSA‡	√		√		

*If haematocrit is > 54%, stop therapy until haematocrit decreases to a safe level, evaluate the patient for hypoxia and sleep apnoea, reinitiate therapy with a reduced dose.
†For patients with osteoporosis or low trauma fracture, consistent with standard of care.
‡After 3 months, perform in accordance with guidelines for prostate cancer screening, depending on the age and race of the patient. Obtain urological consultation under certain conditions.
AEs, adverse events; BMD, bone mineral density; DRE, digital rectal examination; PSA, prostate-specific antigen.

DHT Replacement

• Introduction: Testosteron Exert its effect By 3 Methods

- Directly (Testosterone)
  - Brain
  - Breast
  - Ext. genitalia Enlargment
  - Int. " differentiation
  - Liver
  - Muscle
  - Testis
- Through Conversion To DHT (7% of free T → DHT)
  - Ext. genitalia develpm.
  - Hair follicles (AGA)
  - Sebaceous glands (Acne)
  - Seminal Vesicles (SV)
  - Prostate
- Through Conversion To Estrogen (0.2% of free T)
  - Bones
  - Brain
  - CVS

Site of Conversion To E:
- Adipose Tissue
- Hypothalamus

• DHT differs from T. in:
① More Potent (Two Times)
② No aromatization To Estrogen (Pure Androgen)

• DHT given in following situations: (Pure Androgen needed & Not Aromatized)
① Hypogonadism ② Gynecomastia

② Androgen-deficient Aging ??

No
- Gynecomastia
- ↑ prostate Size
  - E2 → ++ stroma
  - DHT → ++ acini

→ ⓐ less Transformed from Circulation To Prostate.
→ ⓑ lack of Aromatization (Est. + DHT both needed to ↑ prostate Size; Synergism)

7α MENT (Methy nortestosterone)

- under trial
- No reduction To 5α DHT
- More potent than T
- No Gynecomastia or Prostate Enalrgment.

---

2. Each route of T has Favorable & unfavorable Features:

- Oral → Poor or variable absorption
- Parental → undesirable peaks & throughs
- Patches → . limited delivery
  . Allergic Reaction
  . unphysiological High DHT

Most effective & Safest preparations

- Oral < undecanoate / Cyclodextrins / Mesterolone
- Parental < Enanthate & Cypionate
- Topically applied gel.

3. How to limit undesirable effects of TRT on Prostate ??

(1.) use preparations that dot... undergo Conversion TO DHT & Est. e.g MENT

(2) Concomitent use of 5α reductase Inhibitors e.g Finasteride or Dutasteride

NB

(A) Tests currently available for Measurment of Serum Testosterone:

(1) Total Testosterone: (TT)

- Easy
- cheap
- satisfactory for intial Evaluation
- misleading : may be changed ← changes in SHBG

(2) Free Testosterone: (FT)

- Measure the fraction Not bound to { Albumin / Nor SHBG
- Most accurate Index For man androgenecity
- Costly & Need Experience.
- If done by RIA → inaccurate but accurate results Need equilibrium dialysis or ultracentrifugation.

(3) Calculated Free T: (CFT): (معادلات)

- Measures The Free T based on Formula bet. Total T & SHBG & Albumin.
- May be altered by changes in (SHBG).

(4) Bioavailable T.: (BAT) ("بيو")

- include { Free Test. & T. loosely bound To Albumin.
- Provides accurate serum level but Not automated & requires Experience.

(5) Free Androgen Index:

$$FAI = \frac{TT}{SHBG} \times 100$$

- unreliable & Not recommended.

(B) TRT (when T. is deficient) may (improve):

- libido
- sexual function
- Mood
- Cognition
- ms mass & strength
- Bone density.

Anabolic steroids

Testosterone has 2 effects ... attempts were done to dissociate both effects to get benefit from the Anabolic effect and S.E of Androgenic act By chemical alteration of it Molecule

anabolic	Androgenic
(induces ms growth when accompanied By physical Exercise).	

Anabolic steroids

- ✓ So has anabolic effect
  - +ve effect on ms Metabolism
  - Blood Format
  - +ve on Bone Metab
- ✓ no unwanted androgenic S.E e.g. Virilization in women & young children

But at the end → This modified Molecule will have Androgenic effect That may → Infertility

Assisted Reproductive Technologies (ART)

Def: Any medical Technique that interferes with one or more of Mechanisms or barriers that have To be completed before successful fertilization.

Historically:

- First successful AIH → by Hunter 1785 (for Hypospadias pt.)
- IVF → 1978.
- ICSI → 1992.

Classification:

- AIH (Artificial Insemination by Husband)
- IVF (In Vitro fertilizat)
- GMM (Gametes Micromanipulation).

AIH

- Def:
- Indications
- steps (5)
  - Ovulat° Induct°
  - " Monitoring
  - Semen Collect°
  - " processing
  - Insemination Technique.

AIH

Def: Method by ♂ semen is collected by the husband & brought To the female genital Tract by means other than sexual intercourse.

Indications:

4 Infertility Types:

1. Mechanical (Cortal) Infertility < Intromission disorders e.g ED/PD; Ejaculatory " e.g Amy.
2. Immunological Infert.: To < remove antisperm Antib. by passing the Cx Mucus That may Contain Antisperm Antibs.
3. Idiopathic Infertility (Efficacy of IUI is Controversy)*

5 Semen disorders:

1. OAT (but we should have ≥ 5 million "agg" motile sperms after processing). → Morphological NL.
2. ↓ Vol < Hyperspermia (loss of Large No of Sperm)
3. Hypospermia (Failed to form Seminal Prof & Coating (Cx)).
4. ↑ Viscosity & ↓ liquefaction (delayed).
5. ↓ Add. → ① Liquefying Enzs ② Culture Media ③ Repeated Needling
   1 fraction of the ejaculate.
   ↓ add. → Liquefying Enzs: . Amylase . α Chemotrypsin . Hyaluronidase.

Steps of AIH: (٥ خطوات)

Controlled ovarian Hyper Stim.

1. Ovulation induction
   - By COH — either By Clomiphene Citrate, HMG or Both.
   - aim: ↑ No of oocytes against relatively low No of sperms → ↑ Fertilization rate.
2. Ovulation timing
   - How ??
     1. BBT
     2. LH level in Blood
     3. Urinary LH detect Kits (at Home)
     4. Cx Mucous assessment (Insler score)
     5. U/S (gold standard)
3. Sperm collection (باكر طريقة)
   1. Split ejaculate — used in pts. ē Large Volume (مناظر اذن جزيئين) — select 1 portion of ejaculate Contain (Large No of sperms + Good motility). — ZV ↑ Viscosity
   2. Sequential pooled ejaculate — used in pts ē oligoasthenoz. — 2-3 ejaculations over 2-5 hrs → ↑ Sperm Count & motility.
   3. Retrieval in Cases of Anej. & RGE
   4. Cryopreservation
4. Semen processing (٣ طرق) (WSS)
   - Viable, Motile
   - Sperm Washing → ٢ غسلات
   - Selected Motile Viable Sperms → ٣ طرق morphology
     - Swim up Tech
     - Filtration Technique
     - Percoll Centrifugation Technique
   - Stimulated Motile Sperms → ٣ حاجات
     - pentoxifylline
     - caffeine
     - co culture ē epith cell addition

Culture Media
- HTF = human Tubal Fluid
- Hams F-10
- EBSS = Earle's balanced Salt Sol.
- [illegible]
- Human [illegible]

Semen-processing

"[illegible]"

1. Sperm washing.

Sperm separation from seminal plasma to remove harmful substances as:

① PGs → Uterine Contractions → Abort

② ROS → From Leucocytes & damaged sperms

③ decapicitating factors → allow Capicitation & AR. (Acrosome R.)

Method: Liquefied semen + Culture media → Centrifugation

Culture Media

Centrifuge

Semen Pellets → + Culture Media again → Centrifugation

Supernatent Fluid.

Suspended in 0.5 ml Culture Media.

2. Select Motile sperms → Viable → HOS Test; Motile; NL Morphology

Techniques.

diadv.
① Time consuming
② low No of sperms
③ F.R < Per-Coll.

- Percoll Centrifugation Technique.

Sperms + Percoll suspension → NL Sperms (Head & mid piece) have higher density > AbNL → separated from the AbNL & pass to the Concentrated Fractions of Percoll → Selection of NL sperms of Good Motility.

NB: Percoll = Silica + Polyvinyl pyrrolidone. PVP

- Filteration Techniques

. NL motile Sperms have more Flow Through Filterate media (glass wool or Albumin gradient)

. Replaced by Percoll Technique.

- Swim up Technique

. NL motile Sperms Can swim up to media Put on The Surface of Pellets

. Simple & Can Select Sperms of good in Cx penetr. Fertiliz.

③ Stim. of Motile Sperms (all ↑ < motility / fertilizat° ability).

↑ cAMP

Pentoxifylline → ↑ cAMP

Caffeine → as pentoxifyll°

Coculture ē Epith. Cells addition as . Cumulus oophorus . Corona Radiata.

5. Washing Technique.

one of the following 3 Techniques.

Tubal — Peritoneal

IUI

Lithotomy posit°

Ex → Exposed & swabbed ē Culture Media.

Prepared Semen : injected ē a Catheter inside the uterus.

Cx : locked by insemination Syringe for (1 hrs).

ITI

. Fallopian Tube is perfused ē large Vol. of Sperm suspension. (1ml ; IUI 0.5ml)

. Cx → locked by....

. Same results as IUI but may give good results in cases ē (partial) Tubal Obst.

IPI

. Semen susp. is injected into "Douglas Pouch" at time of ovulation.

. Same results as IUI but better in patients ē Cervical Stenosis.

. More Invasive

Efficacy of AIH: May give better Results after ≥ 4 Treatment Trials.

S.E 1. Uterine cramps or inf.

2. Ovarian Hyper Sthm. Synd more in < young ♀ ē PCO / IVF, ICSI > IUI.

"OHSS"

3. Multiple pregnancies if ① young ♀ ② HMG use ③ > 6 Follicles ④ E_2 > 1000 pg/ml.

IVF (&ET)

def. Technique BY which oocytes & prepared Sperms are brought Together in culture Media outside the body & incubated To achieve IVF → then the embryo is Transferred again To the uterus or Tubes

Tip: Sperms + oocytes IVF → Embryo Transfer to The UT.

Indications: ① Immunological infertility.
② Irreversible Tubal obst.

Sperm → we should have ≥ 5 million/ml sperms

With NL < Motility & Morphology

Induct^ / Monitoring → then → oocyte Collect^ < ovulat^

Semen < Collect^ / processing

IVF has a low success Rate so replaced Nowadays by ICSI.

Insemination Technique < IVF / GIFT / ZIFT

Conventional IVF-ET (mixing < sperm / oocyte in Culture Media) & embryo inside the UT.

Modified IVF

GIFT (Gametes Intrafallopian transfer)
- Abd. Laparoscope used to collect 2-4 oocytes + Sperms → mixing & by same Laparoscope introduced into Fallopian Tube where Fertilizat^ occurs (So physiological > Conventional IVF).

ZIFT
- TVUS guided oocyte Aspiration + Sperms (24 hrs in Cub.) → Zygote → Transferred To the Tubes by Laparoscope.

Gametes Micromanipulat

(Both replaced by ICSI) → ① PZD = Partial zona dissect (to facilitate sperm penetration)
② SUZI = Sub Zonal Inseminalion (Inj. 3 sperms in the subzonal perivitelline space)
③ ICSI = ..

ICSI

Def: ... direct injection of 1 sperm inside the oocyte Cytoplasm

The source of used sperms may be: (indications):

① Ejaculated Sperms: in these conditions:

- OAT [Very low Count / Abnormal motility / " Morphology] → AIH
- Abn structure (Immotile Cilia Synd / Globe Zoosp)
- Antisperm Antibs
- sperm ejaculat disorders
- Sperm Fertilizat ⑪ (either < Abnl sperm FTs / Failed IUI or IVF)

② Epididymal sperms: (obst)

- unconnected obst: young Synd & CBAVD
- Failed Correct: TURED, Vasostomy

③ Testicular sperms: (2N)

- Functional Azoosp → Klinefelter, Spermatogenic arrest
- Necrozoospermia

④ Cryopreserved Sperms: (see AIH)

NB: Female indication for ICSI: Failed previous IVF cycles

Technique of ICSI

1. Ovulation ← Induction / Monitoring → Gn / HMG + HCG (but: given when: ① ≥ 3 Follicles ≥ 18 mm diam ② Estradiol > 1000 pg/ml)
2. Oocyte ← Collection: preparation: 36 hrs after HCG by Trans guided or Laparo.
   - ① denuded From cumulus ooph. & Corona Radiata
   - ② Examined For Maturation: ⓐ appearance of germinal vesicles & then its break down (Metaphase I). ⓑ Extrusion of polar body (Metaphase II)
3. Sperm ← Collection (Retrieval) ← from ejaculate / surgical (if Azoo) ; preparation
   - Normal Sperm Retrieval or Aspirat (ESA)
   - Testicular Sperm Retrieval (Extract asp.)

① MESA (MicroSurgical ESA): Uncorrectable cause / Failed Correct

② PESA (Per Cut. ESA): Aspirat: as MESA. but may → Trauma.

③ SPAS (Spermatocele Aspiration): in pts ē Azoo + Spermatocele.

① TESE (Testicular Sperm Extract):
- Sperm Collect during open Biopsy
- Indicat:
  - Functional Azoo.
  - NeCro Zoo.
  - Failed ESA

② TESA (Test. Sperm Aspirat) (TFNA): Test-fine Needle asp. Test. fine Needle asp. (less effective Than TESE of Functional Azoo).

③ RETA: Rete testis Aspirat

Sperm preparation

① Ejaculated sperms → simple washing only
↓ "washing"
Complete processing (d.t very low count).

② Epididymal sperms → 1 mp of Epid. aspirate
+
Culture Media
+
Mineral oil

③ Testicular Sperms (TESE) → Fine Mechanical mincing
↓ à 3 ml Culture Medium
Select ← Viable
← Motile
← Morphology

④ Injection Technique:

• Sperm Immobilization

One single Motile sperm immobilized by rubbing (Aggressive) the tail à Micropipetti → the tail & Hyaluronic acid that activate the oocyte.

- Ratio of sperms / egg:
- N. inseminat : 100 million
- IUI : 5-10 million (Cerl-S)
- IVF : 10.000
- ICSI : 1 sperm

• Oocyte Fixation

- Fixed so that the polar body will be at 6 h or 12 h o'clock

• Sperm Injection

- immobilized Sperms are injected into the oocyte Cytopl at 3 o'clock

↓ at:
- 37 °C
- 5% O_2
- 5% CO_2
- 90% N

& ⊙ Examined for Fertilization ~ 16-18 hrs.

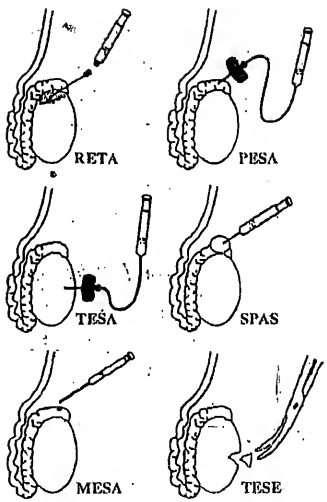


Fig. (34): Methods of surgical sperm recovery for ICSI utilized in men with azoospermia. RETA, rete testis aspiration; PESA, percutaneous epididymal sperm aspiration; TESA, testicular sperm aspiration; SPAS, spermatocele aspiration; MESA, microsurgical epididymal sperm aspiration; TESE, testicular sperm extraction (from a biopsy).

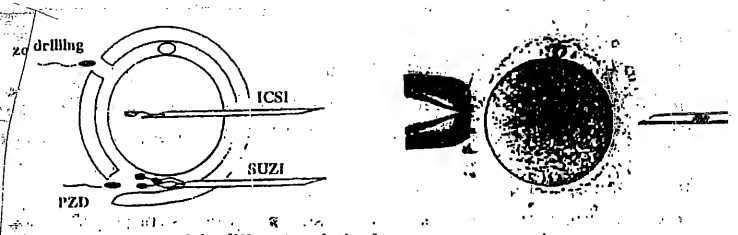
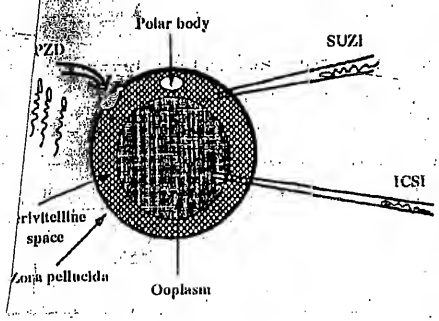


Fig. (3 Graphic presentation of the different methods of microassisted fertilization: zona drilling, partial zona dissection (PZD), subzonal sperm injection (SUZI), intracytoplasmic sperm injection (ICSI).



Risks of ICSI (Complications)

1. Ovarian Hyperstim. Synd:
   - Massive ov. Enlargment
   - peritoneal Irritat
   - ovarian Torsion or Hge.
   - Ascites.
   - pleural effusion.
   - oliguria & ± death.

2. Complications of Ovarian retrieval: < TVUS
   - Hge.
   - Infect.
   - Intestinal & Visceral perforat.
   - Co2 Embolism.

3. Risk of:
   - Abortion.
   - prematurity (& its Complicatn as CP) (Cerebral Palsy.)
   - Ectopic pregnancy.
   - Multiple "
   - Birth defects (d.t passage of Natural barriers of fertilizat & use of severly Abnl sperm for ICSI).

• Results of ICSI:

- in General < FR ↓ ≃ 60% (FR = Fertilization rate)
  PR ≃ 37% (PR = pregnancy rate)
- FR using < Ejaculated Sperms → 60-70%
  Other Retrievals → 55-60%
- Failed Fertilization ± d.t < oocyte Factor: Failed activation
  Sperm ↓ : No sperms for inj. or Morphologically abnl one used. also Spermatid: Early = Round (Failed) late = Elongated ± success (Round Spermatid Nucl. inject: ROSNI)

NB • No significant increase in The Major malformations of children born with (ICSI) Compared To other Children in general populations.

(Sex Chromosm ← RR ...)

• Factors affecting Results of ICSI:

1. Female Age (Most important) [Age > 40 y.]
   it < affects pregnancy rate (PR): ↔ Failed pregnancy, abortion or Anomalies.
   decreases Fertilization rate (FR).

2. Sperm: *ICSI results depends on:
   - ICSI results ē ejaculated sperm better than results ē Sperm retrieval in OA ω is better > Sperm retrieval in NOA.

NB:
Inject using abNL sperm ±
- Failed fertilization
- Abort
- Chromosomal anomalies

- Motile sperm مشي → Immotile
- Morphologically NL sperm مشي → abNL sperm
- Viable sperm (non viable → Failed ICSI)
- Cryopreserved ejaculated sperm تجميد → fresh sperm
- Aggressive Immobilization (by tail Crushing) → better results [d.t better oocyte activat°]

③ oocyte factors:

- Intenh/mp Aggressive oocyte activat → better results of ICSI
- oocyte injury during sperm inject → Failed ICSI

④ Other factors: ↑ Ros in semen → ↓ ICSI results.

NB

- Sperm stress test:

Help Couple Select & ↑ benefit/cost of ART

- اختبار يتنبأ للمتقبل بنتيجة ART
- يعتمد فكرة إن حدوث اختلاف كروية الحيوان المنوي (In vitro) تتعرض بفعل endogenous lipid peroxidat= (That damage Plasma & Acrosome).
- الطريقة: يوضع الحيوان المنوي في أنبوبة اختبار (٤٠) لمدة ٤ ساعات ثم يمرر الحبر الحرة بعد ٥ دقائق وبعد ٤ ساعات (يتم وضعه في الأنبوبة)
- وأقسم = Stress Test (if < 0.75 → Valuable for predict)

. Prenatal ✗
(1) PGD
(2) Amniocentesis
(3) Chorionic Villus Sample

Pre-implantation genetic diagnosis
(Embryo Screening)

- **Pre-implantation genetic diagnosis** (PGD or PIGD) (also known as **embryo screening**) refers to procedures that are performed on embryos (created by IVF) to identify genetic defect prior to implantation. PGD is considered another way to prenatal diagnosis.

NB: 1-- Procedures performed on sex cells before fertilization may instead be referred to as methods of oocyte selection or sperm selection, although the methods and aims partly overlap with PGD.

2- Preimplantation genetic *diagnosis* (PGD) refers specifically to when one or both genetic parents has a known genetic abnormality and testing is performed on an embryo to determine if it also carries a genetic abnormality. In contrast, preimplantation genetic *screening* (PGS) refers to techniques where embryos from presumed chromosomally normal genetic parents are screened for aneuploidy.

test of an embryo

missing or extra chromosomes

3- Preimplantation genetic testing provides an alternative to current postconception diagnostic procedures (ie, amniocentesis or chorionic villus sampling), which are frequently followed by the difficult decision of pregnancy termination if results are unfavorable.

. Indications For doing PGD (Ir candidate For PGD)

. Couples with or with +ve FH of:

. X-linked diseases

. Chromosomal Translocations ~~e.g Down~~

. AD & AR diseases.

. Conditions diagnosed by PGD:

① Sex linked disorders:

. XLD : Incontinentia Pigmenti

. XLR : Hemophilia & Muscular dystrophies

✓ ② Single Gene defect: cystic fibrosis & sickle cell anaemia. defect in structure

③ Chromosomal disorders: Translocations, Inversions & deletions ± → Abortions
→ Genetic Synd. e.g Down & Klinef

Translocat

Numerical (Aneuploidy)

Indications for Preimplantation Genetic Screening

Most early pregnancy losses can be attributed to aneuploidy. Because only chromosomally normal embryos ~~are transferred~~ into the uterus, the risk of first and second trimester loss is markedly reduced. At present, no specific list of indications for preimplantation genetic screening (PGS) is available.

Primary candidates for PGS can include the following:

- Women of advanced maternal age → > 40y. risk of Chromosome Anomalies
- Couples with history of recurrent pregnancy loss
- Couples with repeated IVF failure
- Male partner with severe male factor infertility

Technique

* Biopsy : 3 Types can be used.

Embryo Culture → [1] Cleavage-stage embryo Biopsy

PGD utilizes IVF, where multiple eggs are matured and retrieved; the oocytes are inseminated with a single sperm (ICSI) and the resulting embryos are grown in culture until the 6-8 cell stage (day 3 of embryo development). At this point, the embryo is biopsied with the removal of 1-2 cells. This process does not damage the cells remaining within the embryo.

Oocytes → [2] Polar body Biopsy.
[3] Blastocyst Biopsy.

↓

Then Do:

Aneuploidy: extra or missing chromosome

(1) PCR : diagnose single gene defect.

(2) FISH : Fluorescence In situ Hybridization For Dx of Chromosomal Abnormalities. (X linked dis. Aneuploidy screening.)

(3) CGH : Comperative Genomic Hybridization

NB : In sex (X) linked diseases → Select of Sex of Embryo.

Sperm (Y) & (X) ...

X Sperm Carry 3% DNA >

Cryopreservation (Sperm banking)

(عملية معقدة احكم)

- **Def:** Technique depends on prophylactic Semen Collect & Cryopreservation for fututure ART.

- **Indications:** In the following conditions that may carry arisk of loss of fertility:

1. Testicular Tm.
2. Leukemia & Lymphoma before < Chemo & Radio ++
3. Before vasectomy.
4. after Vasovasostomy.
5. Oligozoo (banking of pooled split ejac.)
6. Cryopreservation after sperm retrieval (For future ICSI)
7. storage of several donors semen carrying desired certain genetic (chic)

- **Procedure:** 2 Steps of Freezing (to avoid Sperm injury)

In liquid Nitrogen.
1. 1st step → Freezing To -8°C
2. 2nd " : → " " -196°C

- **Note:** Freezing rate should be:
  - 10°C/min (أبطأ شوية)
  - 1-2°C/min (أكتر طبيعي)

Note That

- Slow Freezing → Cell dehydrat^ ē extracellular electrolyte conc. → Cryo injury
- Rapid freezing → Cell disruption
- Thawing rate should be = Freezing rate e.g. 1-2 °C or 10 °C/min

NB Thawing protocol

- Slow (20-25 °C air) (حرارة الجو)
- quick (بالحمام)

• الإضافة معادن في السمن

A. Cryoprotectants aim

① ↓ Electrolyte Conc.

② ↑ memb. stability

e.g. Glycerol, DMSO

B. Extenders: aim

① optimize osmotic pressure

② " pH

③ provide Energy

e.g. egg yolk.

disadv. ↓ sperm quality (specially Motility) why ??

d.t ↓ HSP 90

• المرجع لتصحيح دماغه:

انظر المحرين 269 طبعة (٢٠٠٨)